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Full Length Research Paper

Ethanol production from *Washingtonia robusta* fruits by using commercial yeast

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In this study, ethanol production from fruits of *Washingtonia robusta* was investigated. Effects of different contact times (1 - 24 h), pH (2 - 12) temperature (20, 30, 40, 50˚C) and autoclave pretreatment (121˚C, 10 min, 1.2 atm) were also studied to improve the solubility of reducing sugar in fruits. Baker's yeast (*Saccharomyces cerevisiae*) was used for fermentation of that reducing sugar. Results showed that solubility of reducing sugar increased with increasing contact time. Optimal pH and temperature for solubility of reducing sugar were 6.8 - 7.2 (deionized water) and 50˚C. HPLC analysis showed that samples contained only glucose and fructose as a sugar source. Autoclave pretreatment protected the samples from contamination and increased the reducing sugar concentration up to 105 g/L. Ethanol production reached up to 25 g /L at the end of eight days by using commercial yeast. Bioethanol yield was found to be 71.42 ± 1.4 g ethanol/kg fruit.

Key words: Ethanol, fruit, reducing sugar, *Washingtonia robusta*.

INTRODUCTION

There has been increasing interests in conversion of biomass to fuel grade ethanol for many years due to variety of reasons including alternative green energy sources, the rise in oil prices, minimizing greenhouse gas (GHG) emissions caused by the use of fossil oil and others (Huang et al., 2009; Demirbas, 2005).

Ethanol is fermented from sugars, starches and cellulosic materials. Production of ethanol by fermentation from renewable carbohydrate materials for use as an alternative liquid fuel has been attracting worldwide interest. The current technology in industry is able to convert carbohydrates from dedicated crops such as corn, wheat, sorghum, potato, sugarcane, sugar beet and cassava to ethanol (Luo et al., 2009; Mohanty et al., 2009). However, the land use requirement of such an application causes the competition with food and nature, which has become the main driving force of the development and implementation of advanced process technologies to produce ethanol from low value agricultural co-products/ residues or wastes (Luo et al., 2009; Mielenz, 2001). With this point of view, fruits of different plants, which have already easily convertible sugar, can also be an alternative low-cost feedstock.

Washingtonia robusta (Mexican Fan Palm, Petticoat Palm), the Skyduster Palm is a fast-growing palm with a thick, reddish trunk and big, dark green leaves. Long inflorescences of small fleshy flowers are produced in the late spring, followed by black-brown berry-like, small fruits that have a thin, sweet pulp that tastes somewhat like dates or butterscotch. Each fruit contains a single seed. *W. robusta* fruits have high fermentable sugars. To the best of the researchers' knowledge, there is no scientific study on the ethanol production and reducing sugar solubility from *W. robusta* fruit coats up to now. The aims of this study are to use this low-cost material to obtain reducing sugar and convert this sugar to a valuable product, ethanol.

MATERIALS AND METHODS

The *W. robusta* fruits, which grown in Mersin, Turkey, harvested in September 2007 and separated from other parts of tree. It was stored in plastic bottles at +4°C. Fruits used in this study were shown at Figure 1.

Abbreviations: RSC, Reducing sugar concentration; **DW,** deionized water; **STA,** static condition; **AGT,** agitated condition; **AU,** autoclave treatment.

Figure 1. *W. robusta* fruits used in present study.

Effect of contact time to increase reducing sugar solubility

W. robusta fruits were placed in 100 mL flasks and added deionized water to reach 50 g /L final concentration. Flasks were put into an incubator at 30°C for 24 h. Fifteen samples (3 mL, each) were collected at an interval of 24 h. These samples were centrifuged (6000 rpm, 10 min) and filtrated (through 0.45 µm pore size membrane filter) and than filtrates were used for reducing sugar analysis.

Effect of temperature to increase reducing sugar solubility

To investigate the effects of temperature, static experiments were performed at 20, 30, 40 and 50°C for 50 g/L fruit-deionized water mixture. Collected samples (3 mL) were centrifuged (6000 rpm, 10 min) and filtrated (through 0.45 µm pore size membrane filter) after 24 h contact time, and filtrate was used for reducing sugar analysis.

Effect of pH to increase reducing sugar solubility

The pH of media was adjusted to 2.0, 4.0, and 6.0 with HCl and to 8.0, 10.0 and 12.0 with NaOH. Fruit-deionized water mixture (pH 6.8 - 7.2) was also used as a control group. Initial fruit concentration in pH adjusted media was fixed to 50 g /L. Static (STA) and agitated (AGT) experiments (150 rpm) were performed at 50°C for 24 h contact time.

Effects of autoclave (AU) treatment to increase reducing sugar solubility

Same sets of experiments were also performed to investigate the effects of autoclave treatment. Flasks, which contained 50 g/L fruit-deionized water mixture, were autoclaved at 121°C during 10 min to protect from contamination and to enhance sugar concentration. After treatment, samples were centrifuged and filtrated. Filtrate was used for reducing sugar analysis.

Fermentation media

Commercial baker's yeast *Saccharomyces cerevisiae* was used for inoculation. Synthetic medium, which consisting of malt extract (3 g, from Sigma), yeast extract (3 g, from Fluka), peptone from animal proteins (5 g, from Fluka), technical grade glucose (5 g, from Merck) and deionized water (1 L), were prepared in Erlenmeyer flasks (500 mL) and dried *S. cerevisiae* (5 g) was added into this media. The inocula were incubated during 12 h at 30°C and 150 rpm in an orbital shaker. For inoculation, 5 mL yeast media were added into flasks containing 105 690 mg/L reducing sugar obtained from autoclaved fruits. The fermentations were carried out at 30°C and pH was not controlled. Samples were collected every two days (250 mL), centrifuged (6000 rpm, 10 min) and filtrated (0.45 µm pore size membrane filter). Filtrate was used for reducing sugar and alcohol analyses.

Analytical methods

Sugar analysis was performed by HPLC (RID detector, Intersil-NH_2 column, 5 µm 4.6 mm ID x 250 mm, column temperature 40°C, injection volume 20 µm, and speed 1 mL/min). Alcohol was distilled and measured using a Gay-Lussac alcoholmeter and ethanol productivity was calculated as the gram of ethanol per liter of liquid volume produced every two days (Kourkoutas et al., 2003). The ethanol yield was expressed as g ethanol/kg fruit. Total reducing sugar was analyzed using the DNS (dinitrosalicylic acid) method as reported by Kadam et al. (2006) after sample pH was adjusted at 7.0. All experiments were performed in triplicate.

RESULTS

Reducing sugar concentration obtained from *W. robusta* fruits increased with increasing contact time (Figure 2) and reached approximately 11000 ± 104 mg/L after 6 h

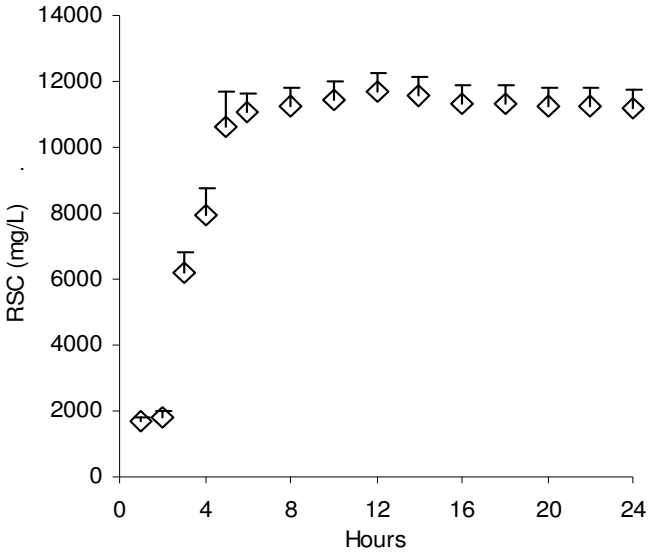

Figure 2. Reducing sugar concentration (RSC) of fruits (50 g/L) in distilled water (30°C).

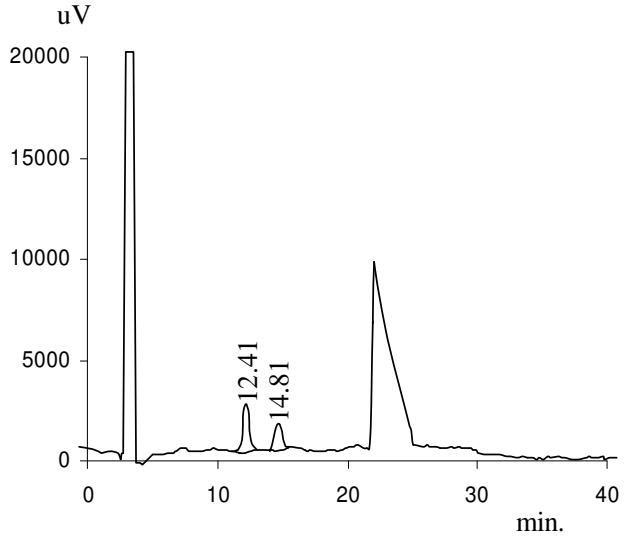

Figure 3. The RID chromatogram of the sample (Intersil-NH2 column, column temp. 40°C, injection volume 20 µL sample), 1; fructose 2; glucose.

without any pretreatment. HPLC analysis showed that these samples contained fructose and glucose as reducing sugars (Figure 3).

Effects of temperature

Figure 4 shows that the reducing sugar concentration was increased steadily with time at different temperature. Surprisingly, temperature values at 20, 30 and 40°C were observed to be less effective for solubility of reducing sugar and almost similar curves were determined at 30 and 40°C. Concentrations for these temperatures were approximately 11186 ± 114 mg/L and 11148 ± 103 mg/L, respectively. A solubility of 14000 ± 144 mg /L of reducing sugar was obtained at higher temperature level (50°C).

Effects of pH

Figure 5 shows effect of pH on solubility of reducing sugar of fruits. Reducing sugar concentration increased with increasing pH up to 7.0 ± 0.2. Sugar solubility was found between 4 000 - 8 000 mg /L in base treated media. However, acid treated samples had higher reducing sugar concentrations than base treated samples. Acid treatment changed visibility of media in AGT and AU conditions and it turned blackish-brown colour at pH 2 and 4. However, visibility of base treated media and STA condition were not affected. Maximum reducing sugar (18 752.74 ± 162 mg/L) was obtained by AU treated samples at 121°C for 10 min. To enhance reducing sugar concentration before ethanol production step, up to 350 g fruits were added in Erlenmeyer flasks containing 1 L distilled water. Results showed that concentration of reducing sugar increased with increasing fruits weight in the media and reached 105 g/L after AU treatment (Figure 6).

Ethanol production

Baker's yeast and stocks obtained from AU treatment were used for ethanol production. After inoculation, samples were collected at two-day intervals and analyzed after centrifugation. Alcohol was produced linearly during the incubation (Figure 7) and however, reducing sugar concentration was also consumed linearly by yeast. Maximum alcohol production was 25 g/L on 8th day and bioethanol yield (g ethanol/kg fruit) was found 71.42 ± 1.4.

According to municipal report in Mersin, 13500 trees have been grown in Mersin. Average of fruits collected from each tree was found 30 ± 2 kg. These results indicated that it can be possible to collect about 390 tons fruits each year. Bioethanol yield could reach approximately 28.92 tons ethanol/year only from *W. robusta* fruits collected from Mersin border and using only commercial baker's yeast as microorganism is the main contributor to the increased costs of cellulosic ethanol production.

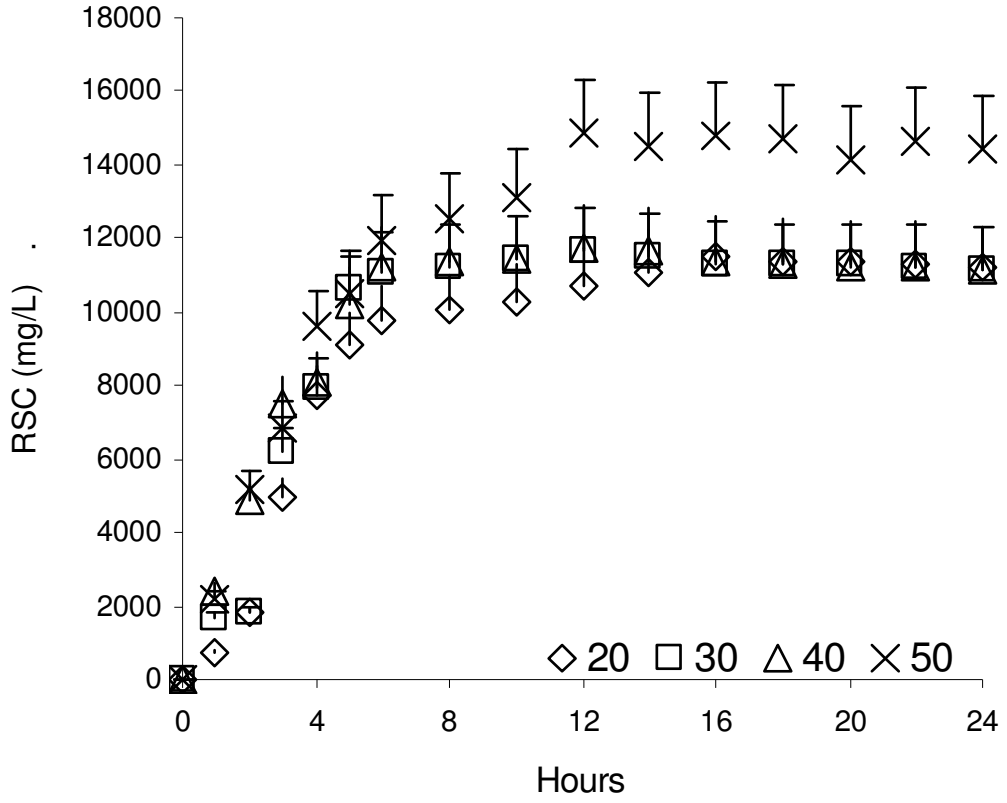

Figure 4. Effect of temperature to increase reducing sugar solubility of fruits (50 g/L).

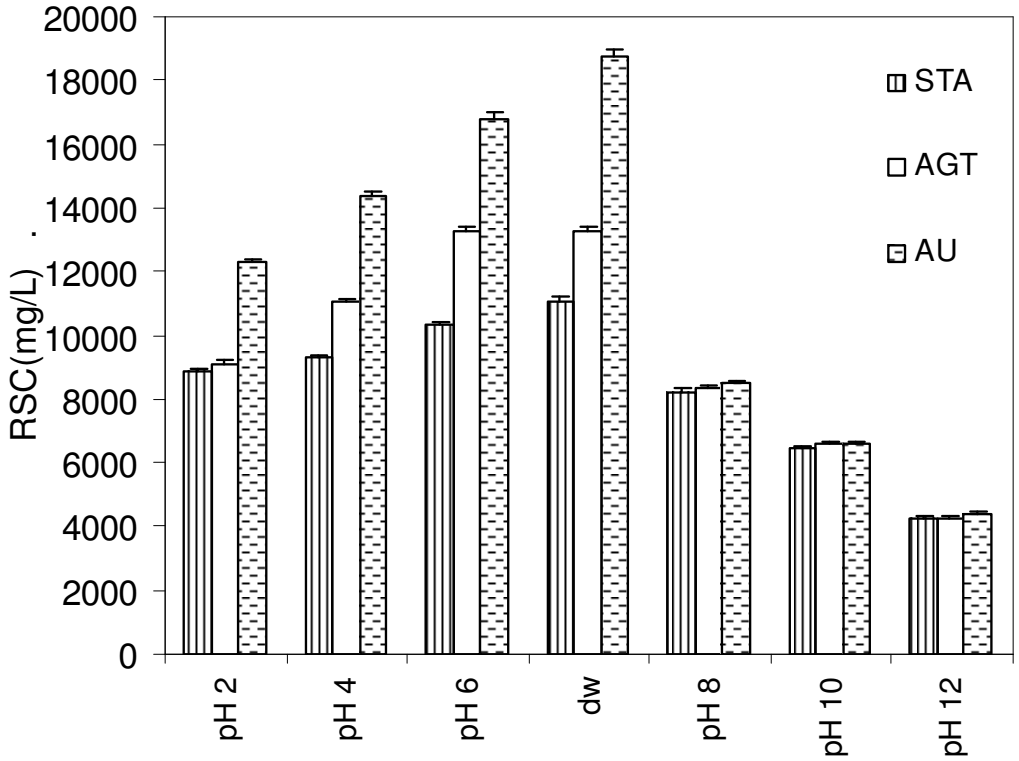

Figure 5. Reducing sugar concentration of sample at different pH values (30°C on AGT and STA condition after 24 h and after AU treatment at 121°C, 10 min).

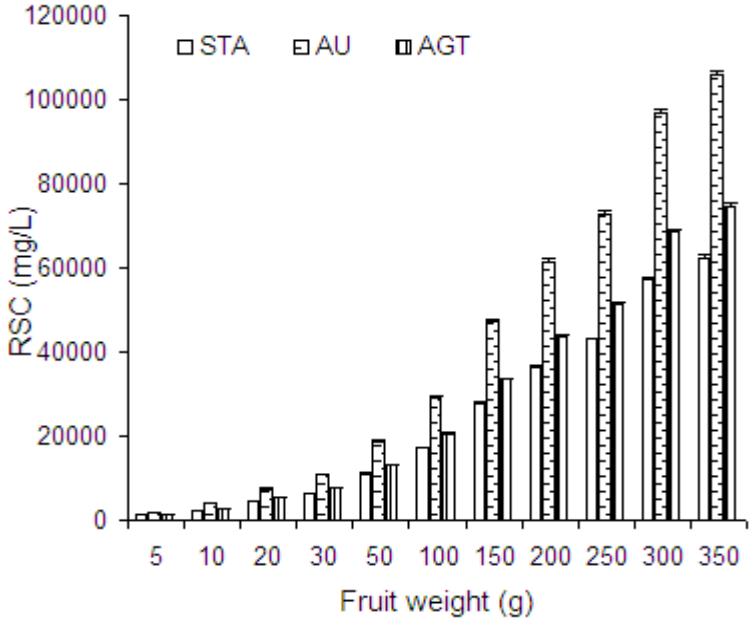

Figure 6. Effects of fruits amount on reducing sugar concentration (RSC) at 30°C on AGT and STA condition and AU treatment.

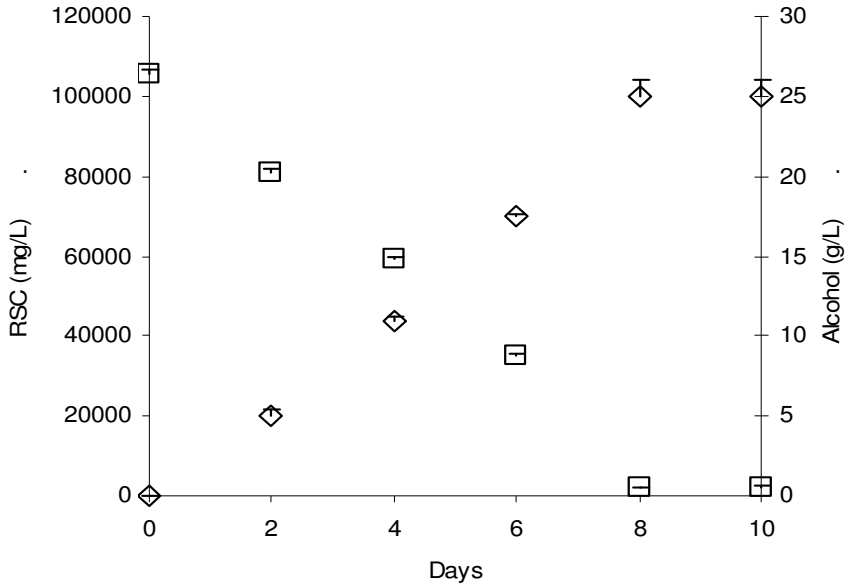

Figure 7. Ethanol production from fruits of *W. robusta* (◊: alcohol production, □: reducing sugar concentration- RSC).

DISCUSSION

The feedstock pretreatment is the main contributor to the increased costs of the ethanol production process.

Several physical, physical-chemical, chemical and biological processes have been developed for the pre-treatment of lignocellulosic and starch-based feedstocks (Sun and Cheng, 2002). However, ethanol production from *W. robusta* fruits does not need extra pretreatments such as acid/base treatment, neutralization and detox-ification.

There is a growing interest worldwide to find out new and cheap carbohydrate sources and the short way to obtain fermentable sugars for production of bioethanol. In present paper, the pH level of 6.8 - 7.2 and temperature of 50˚C were found optimum for solubility of fermentable

sugar from fruits. Autoclave treatment provides two major advantages; to enhance reducing sugar analysis in short time and to protect samples, containing high and easily convertible sugar to ethanol, against microbial attack.

Bioethanol production from mahula (*Madhuca latifolia* L.) flowers by solid-state fermentation was reported by Mohanty et al. (2009). These researchers reported that optimum concentration was pH 6.0 and 30˚C. Ethanol yield (g per kg) reported from other sources such as cane molasses (Reed, 2002), dried sweet potato chips/flour (Woolfe, 1992; Yu et al., 1996) and cassava chips/flour (Balagopalan et al., 1987; Ward and Ray, 2006) were 265 - 272, 280 - 320 and 420 - 450 g per kg, respectively. In present study, ethanol yield was calculated as 71.42 ± 1.4 g ethanol /kg fruit. However, it can be possible to enhance this yield by using different microorganisms such as *Zymomonas mobilis* (Claassen et al., 1999), *Saccharomyces bayanus* (Castellar et al., 1998), *Saccharomyces pastorianus* (Fujii et al., 2001), *Kluyveromyces fragilis* (Szambelan et al., 2004) or genetically modified microorganisms (Ostergaard et al., 2000), or different techniques such as immobilization.

Swain et al. (2007) reported a new material Mahula flowers (*M. latifolia* L.) for bioethanol production using free and immobilized yeast. They also reported that flowers had fermentable sugar like *W. robusta* fruits used in the present study. Bioethanol production was reported 193 - 148 g/kg for free cells and 205 - 152 g/kg for immobilized *S. cerevisiae* (strain CTCRI). According to literature data, ethanol production yields from cassava and sweet potato (Ray and Ward, 2006) are higher than our results (*W. robusta* fruits). However, energy demand for liquefaction and saccharification of starch from these products and the cost of the process are really high.

Conclusion

Energy crisis forces researchers to find new and low cost sources for ethanol production. In this study, *W. robusta* fruits were used for ethanol production. Experimental results showed that fruits had reducing sugars such as glucose and fructose. Samples treated with acid or base were found to be ineffective to solubility of fermentable sugar when compared to untreated media. Solubility of these sugars in deionized water reached a maximum level after autoclave treatment. Ethanol yield was approximately 28 tons ethanol/year when using commercial baker's yeast and *W. robusta* fruits collected from Mersin City, Turkey. However, it can be possible to increase this yield by using genetically modified microorganisms for ethanol fermentation.

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